VAN GIESON, (I)

Laboratory Notes of Technical Methods for the Nervous System.

BY

IRA VAN GIESON, M. D.,

FIRST ASSISTANT AT THE LABORATORY OF THE ALUMNI ASSOCIATION OF THE COLLEGE OF PHYSICIANS AND SURGEONS NEW YORK.

REPRINTED FROM

The New York Medical Journal

for July 20, 1889.

Reprinted from the New York Medical Journal for July 20, 1889.

LABORATORY NOTES OF TECHNICAL METHODS FOR THE NERVOUS SYSTEM.

By IRA VAN GIESON, M. D.,

FIRST ASSISTANT AT THE LABORATORY OF THE ALUMNI ASSOCIATION OF THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK.

METHODS OF OPENING THE BRAIN AT AUTOPSIES AND FOR GENERAL DISSECTION OF THE BRAIN.

1. *Meynert's Basal Section of the Brain.*—The ordinary method of opening the brain at autopsies by slitting open the corpus callosum, laying aside the hemispheres, and then making longitudinal and transverse incisions, renders it difficult to handle the brain without tearing it, and, after hardening, the pieces can not be fitted together well, so that the lesions may be accurately localized in conjunction with the microscopical examination. Meynert's section is much more valuable for the purposes of localization after hardening, and is recommended for general use at autopsies and as an instructive method for demonstrating to students the coarser anatomy of the fresh brain. With some modifications of Meynert's original plan, the section is made as follows:

Resting the brain upon its hemispheres and lifting up the cerebellum, the pia mater and vessels are cut above the corpora quadrigemina and around the crura and inner

margins of the temporal lobes on either side until the middle cerebral arteries are reached. The incision of the pia then follows the middle cerebral arteries into the Sylvian fissures, and passes to the terminations of the posterior branches of the Sylvian fissure. As the incision of the pia follows in this way, the boundaries of the temporal lobes, the temporal convolutions should be gently separated from the neighboring convolutions and from the base.

The apices of the temporal lobes are then lifted up, and, resting the knife flat on the base, their junction with the base is cut, until the descending horn is opened. Then inserting the knife into the descending horn, the incision passes outward and backward to within an inch of the apex of the posterior horn, or even to its extremity, severing the junction of the occipital and temporal convolutions on the lateral surface of the brain. During this manipulation the cornu Ammonis and posterior pillars of the fornix should not be cut. The temporal lobes are thus freed from the base and folded outward and backward over the occipital lobes, giving access to the island of Reil (Fig. 1). The operculum is then pulled well outward to completely expose the convolutions of the islands, and a slightly curved transverse incision (*a*, *a*, Fig. 1), with the convexity directed frontally, is made connecting the anterior sulci of the islands on both sides. This incision should be deep enough to pass through the anterior horns of the ventricle to the corpus callosum.

The cerebellum is then lifted up with the left hand, and placing the knife in the ventricle at *c*, Fig. 1, and passing it beneath the rounded posterior extremities of the optic thalami, the internal capsules on either side and the anterior pillars of the fornix and septum lucidum are cut from behind forward without injuring the basal ganglia. As the internal capsules are being cut, the cerebellum is gradually

rolled over forward on the base, in order to lift up the basal ganglia from the corpus callosum. A square piece—"the

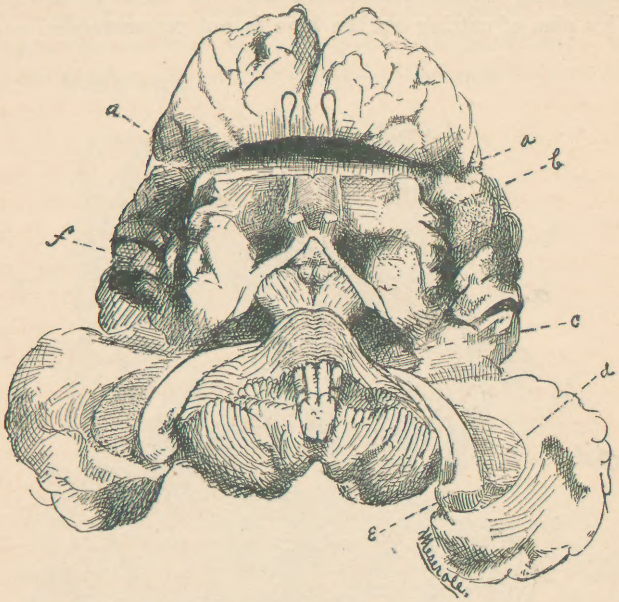

FIG. 1.—Drawing of the brain, showing how the brain axis is exposed before it is cut out. *a*, anterior sulci of the islands of Reil; *b*, convolutions of the island; *d*, descending horn in the reflected temporal lobe; *e*, cornu Ammonis, with the posterior pillar of the fornix passing from it into the ventricle; *f* passes across the convolutions of the operculum to the cut surface of the junction of the temporal lobe with the base.

basal piece" (Fig. 2)—is in this way cut out of the base. The fornix is left lying on the corpus callosum.

When the brain is soft, and in children's brains and in cases where the basal ganglia are injured by hæmorrhage, the above-described method of removing the basal piece is modified by putting the index and middle fingers of the left hand through a transverse incision of the base (Fig. 1, *a*, *a*)

into the anterior horns of the ventricles and raising the basal piece, while the internal capsules, anterior pillars of the fornix, and septum lucidum are cut from before backward. This plan of cutting out the base is not recommended ex-

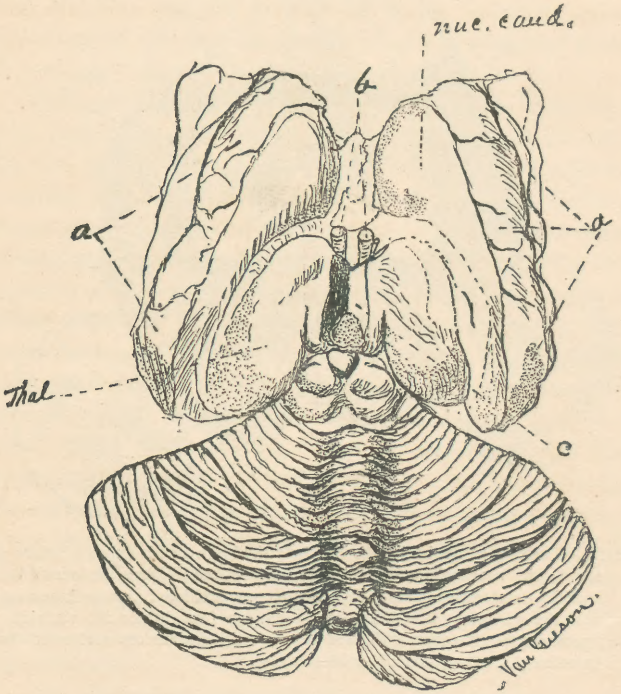

FIG. 2.—Ventricular surface of the basal piece or brain axis. *a*, *a*, fibers of the internal capsule cut transversely as they enter the basal gangiia; *b*, anterior pillars of the fornix cut across; *c*, third ventricle; *nuc. caud.*, caudate nucleus; *thal.*, optic thalamus.

cept when the brain is very soft, because the introduction of the fingers into the ventricles is apt to damage the ependyma.

The advantage of this plan of opening the brain is that it uniformly separates the organ into two great anatomical subdivisions. The square basal piece—the *brain axis* (Fig. 2)—includes the island, basal ganglia, internal capsules, crura, pons, medulla, and cerebellum. The remaining piece —*the brain mantle of Burdach* (Fig. 3)—includes the con-

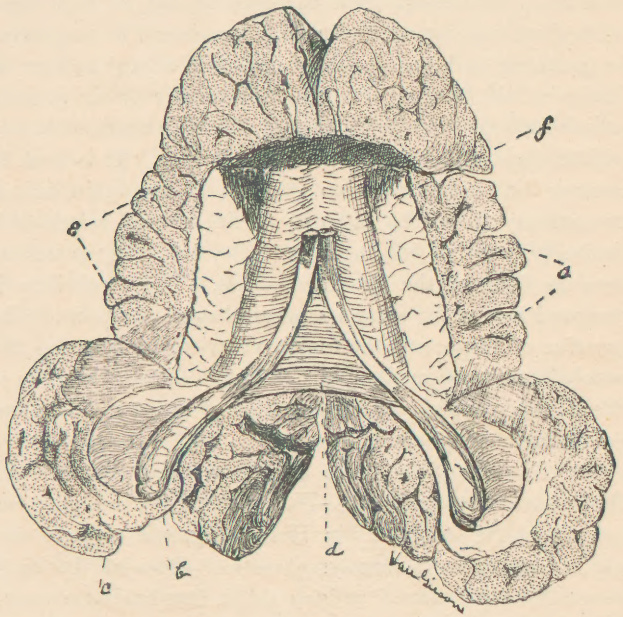

FIG. 3.—Ventricular aspect of the brain mantle. *a*, internal capsule; *d*, posterior border of the corpus callosum; *b*, cornu Ammonis; *c*, descending horn; *e*, operculum; *f*, anterior pillars of the fornix cut transversely.

volutions, corpus callosum, fornix, and cornu Ammonis. This method also exposes the internal capsule (Figs. 2 and 3) advantageously for the localization of lesions in the internal capsule, because its fibers are cut transversely between their entrance into the basal ganglia and the centrum ovale.

Both these pieces may be hardened *en masse* by making transverse incisions about one half to three quarters of an inch apart in the basal piece, and both transverse and longitudinal incisions into the white matter of the larger piece not quite reaching to the pia mater, which serves to hold the convolutions together for the purposes of localization after hardening. The incisions should be kept open with absorbent cotton, and each piece should be suspended in gauze or laid on absorbent cotton in a large volume of Müller's fluid (five or six times the volume of the pieces), which is kept cool and changed on the second, fifth, and eighth days, and again in the third week. It is best to remove the cerebellum from the rest of the brain axis at the autopsy unless the integrity of its pontial relations is desirable, so that in hardening the fluid may penetrate into the floor of the fourth ventricle. The cerebellum is removed by cutting away its crura close to the pons. The practice of inspecting the fourth ventricle by splitting the cerebellum in the median line is apt to distort the floor of the fourth ventricle by the traction of the two divergent cerebellar hemispheres.

2. *Division of the Brain into Transverse Segments for Examination at Autopsies.*—This method is used quite frequently at the laboratory in Heidelberg, and consists simply in making serial transverse vertical slices of the brain about three quarters of an inch thick, first having removed the cerebellum and pons by cutting the crura cerebri transversely. When the slices have been hardened they may be easily fitted together for the detailed localization of the lesions. This method is good for the preparation of certain museum specimens and for lantern demonstrations of tumors or cortical lesions by stained sections of the entire brain, made with the Gudden microtome.

3. *Preparation of the Brain for the Dissection of the*

Association, Commissural, and Projection Fibers, by the Cleavage Method.—The pia mater is carefully removed from the convolutions, and the brain—suspended in gauze in forty-per-cent. alcohol, which is renewed on the third day—is turned on a new surface each day to avoid flattening. No incisions are made except a small one through the infundibulum to let the fluid into the lateral ventricles. At the end of the first week of hardening, the alcohol is replaced by sixty-per-cent. alcohol; at the end of the fourth week this is replaced by eighty-per-cent. alcohol, and then, for three weeks or a month, the brain is left in strong alcohol. With this treatment the bundles of fibers of the white matter become tough and elastic, and a particular tract may be isolated by pushing and cleaving aside the surrounding structures with a scalpel handle. The same method of hardening may be used if dissociation of the tracts is to be practiced in some particular portion of the brain, such as the brain axis or brain mantle.

As an example of how a system of fibers may be dissected out when the brain is successfully hardened in this way, the path of the motor tract in the brain axis is selected. The brain axis (Fig. 2) is hardened as described above, and the pyramids of the medulla are loosened from the olivary bodies, and from each other in the median line to their decussation, with the handle of a scalpel. The superficial transverse fibers of the pons are cut in the median line and pushed away from the pyramids with the scalpel handle inserted in the groove between the inferior pons border and medulla, until they are reflected as two flaps on the middle cerebellar crura. The pyramids are now exposed between the two sets of transpontial fibers, and, when freed from a few interlacing pons fibers, they may be picked up with the forceps and followed up through the middle thirds of the crura into the internal capsules. This method

is exceedingly valuable for teaching purposes, and a series of these cleavage dissections is the best way of giving students a clear idea of the various association, commissural, and projection systems. Plates 18 to 22 of Meynert's "Psychiatry," translated by Sachs (Putnams, New York, 1885), and Plates 28 and 30 in Edinger's "Zehn Vorlesungen über d. Bau d. nerv. cent. Organe," 1885, may be used as guides for other dissections of the different systems composing the white matter.

NOTES ON THE HARDENING OF THE BRAIN.

Müller's fluid does not preserve the ganglion cells as perfectly as bichloride of mercury or alcohol, but it preserves the nerve fibers and neuroglia well; it gives the proper consistence for cutting sections, and all the important methods of staining the nervous system depend upon bichromate hardening. The obstacle in getting perfect hardening results with Müller's fluid is the great impermeability of the cerebral tissues. To get the best results in hardening the pieces should not be more than one to two cubic centimetres in volume, and should be left in the fluid at the temperature of the room until they have a dark-brown color, and sections cut by hand do not wrinkle when placed in water. This may take four or five months or longer, and the blocks should be frequently tested to guard against their becoming brittle. Rapid hardening in the thermostat is not often used in this laboratory; it is not so good as slow hardening at low temperatures, or at the ordinary temperature of the room. In hardening with heat, the superficial portions of the specimens become hardened too rapidly, and become dense and prevent the fluid from gaining access to the central portions of the blocks. If rapid hardening is necessary, it is much better to renew the fluid frequently after the first two or three weeks of the

hardening, and at each renewal to gradually increase the strength of the bichromate-of-potassium solution up to five per cent. *Erlicke's solution* hardens the superficial portions of the blocks more rapidly than Müller's fluid, and consequently does not preserve the central portions so well. It also has a greater tendency to make the specimens brittle. The bichromate solution should always contain camphor (a piece of the size of a pea to each pint of the fluid) to prevent the development of micro-organisms.

When specimens have become brittle it is very difficult to get them into good condition for cutting. A prolonged immersion of the specimens in equal parts of glycerin, alcohol, and water reduces the brittleness somewhat, but the best results have been obtained by soaking the blocks for twenty-four or forty-eight hours in a fifty-per-cent. or stronger aqueous solution of ammonia. A twenty-four-hours' immersion in peroxide of hydrogen also tends to make the specimens less brittle. When very large segments of the brain are to be preserved for microscopical examination it is best while hardening to keep the bichromate solution cold for the first month or two in a refrigerator.

If specimens are not thoroughly hardened in Müller's fluid, the subsequent permanent preservation in strong alcohol does them considerable injury. The alcohol slowly dissolves out the chrome salts and changes the myelin. In the course of a year or two cholesterin crystals form in the alcohol, and minute cavities and vacuoles appear in the specimens and they do not stain well. If for any reason it is necessary to examine specimens before they are thoroughly hardened, it is best to keep the material for permanent preservation on the shelves in water containing camphor, or dilute, thirty to forty per cent., solutions of alcohol. In the laboratory even thoroughly hardened material is not kept permanently in strong alcohol. Seventy or eighty per cent.

is used, and in certain cases water. Well hardened material kept in water has a tendency to become brittle in the course of years. The structure of the neuroglia never shows so well, and carmine staining is never so successful in sections of material which has been placed in alcohol as in sections cut from specimens while in Müller's fluid, or kept in water slightly tinged with Müller's fluid.

DIVISION OF THE SPINAL CORD INTO ITS ANATOMICAL SEGMENTS FOR MICROSCOPICAL EXAMINATION.

The portions of the cord from which each pair of spinal nerves arise are termed the corresponding segments of the cord, and it is desirable to use this well-defined anatomical segmental structure of the spinal cord as a basis for the localization of lesions in the microscopical examination. We can then know exactly from what part of the cord a given section is taken.

The cord may be hardened with the dura mater attached, but is distorted less if the dura is removed. At intervals of little less than half an inch the cord should be traversed by transverse incisions not completely severing the cord, in order that the pia mater may hold the pieces together in their serial order. The cord is then rolled up in a loose spiral and laid in Müller's fluid on absorbent cotton. After the subsequent alcohol hardening the attachments of the nerve roots form a guide for the division of the cord into its proper segments.

To distinguish the spinal segments after they have been taken from their serial order in the hardened cord, I have found it most convenient to mark them by sticking a hot teasing needle in the white matter of one half of the upper surface of each segment. This burns a permanent little hole in the segment, and, by making notes of the different positions of the needle-holes in the different segments, a num-

ber of blocks from the cord may be carried through the celloidin imbedding process together and easily identified. If imbedded on pieces of white wood, the number of the segment or portion of the spinal segment to which the imbedded specimen belongs may be written on the wood in pencil. In the sections the needle-hole also indicates the right or left hand side of the cord. When it is necessary to examine the spinal nerve roots, these may be reflected against the corresponding segment and tied in place with thread, and then the segment with its roots is imbedded in celloidin, so that transections of the upper level of the segment will include transections of the corresponding nerve roots. The lumbar and sacral segments are so short that the entire segment may be mounted on the block of wood; but in the cervical and dorsal regions it is more convenient to use only the upper thirds or quarters of the segments for mounting.

OSMIC-ACID STAINING OF LARGE NERVE TRUNKS.

Most of the human peripheral nerve trunks are so thick that osmic acid does not penetrate into them when stretched out in the ordinary way on a bridge, as advised by Ranvier. To stain the human posterior tibial or sciatic nerves affected with peripheral neuritis, the nerve trunks are cut with sharp scissors into segments a little less than half an inch long. One extremity of the segment is held with a forceps, and with a second forceps the protruding funiculi at the other extremity are gently pulled out of their lamellar sheaths and laid in the one-per-cent. osmic-acid solution for twenty-four or forty-eight hours. This manipulation cracks the myelin sheaths transversely somewhat, but the osmic acid penetrates well among the fibers, and stains them quite uniformly. Osmic-acid nerves are kept best in glycerin.

NEW STAINING METHODS FOR THE PERIPHERAL NERVES.

1. *Dilute Aqueous Solution of Basic Fuchsin for staining the Connective Tissue of the Peripheral Nerves in the Fresh Condition.*—A slender funiculus of a human nerve, pulled out as described above, or a bit of the sciatic of a frog or mouse (not over half an inch long), is laid upon a slide for twenty to thirty seconds, until its surface begins to stick to the glass. (If the bit of nerve is very wet, it is moved about on the slide until some of its surfaces begin to adhere to the glass.) Then, with two teasing needles, the nerve is rapidly rolled or pulled away from the adherent portion, so that it is spread out into a thin film on the glass. Before it dries—this is to be avoided by breathing on the specimen—a drop or two of an aqueous solution of basic fuchsin, such as is used for bacterial staining (one drop of a saturated alcoholic solution of basic fuchsin in eight or ten c. c. of distilled water), is placed on the specimen. The slide is inspected with the low power, and in two or three minutes the nuclei of the endoneurium will be stained. The specimen may be mounted and studied in the staining fluid, or the dye may be washed with water; the stained nerve is dried in the air on the slide and permanently mounted in balsam. In drying in the air, the endoneural cells are not perceptibly distorted. Sometimes the dye penetrates through the constrictions of Ranvier for a slight distance between the axis-cylinder and periaxial sheath. I have found this method of service in studying the endo neurium in normal nerves, in experimental nerve lesions, and in peripheral neuritis in the human nerves.

2. *Acid Fuchsin for staining Isolated Hardened Nerve Fibers.*—Nerves are hardened from three to five weeks in Müller's fluid, and then, after washing out some of the Müller's fluid in water, for a week in strong alcohol. A

slender strand of fibers, about half an inch long, is teased very finely in water on a slide. The water is then removed, and the specimen covered with a drop of a saturated aqueous solution of acid fuchsin (Grübler's) for two to five minutes. The teased fibers are washed in water, then thoroughly in two alcohols, cleared in oil of cloves, and mounted in balsam. The axis-cylinder, neurilemma, constrictions, incisures of Schmidt, neurilemma nuclei, and branching cells between the fibers have a distinct red color. In successful preparations the incisures show very distinctly. This method is so simple and efficient in demonstrating all the details of the nerve fiber, except the medullary sheath, that it is recommended for staining specimens for classes in normal histology.

The most convenient nerves to prepare for this method are the spinal nerve roots, because they can be teased more readily than the peripheral nerves, which contain more connective tissue. When nerves have been hardened for a short time (three to eight days) in Müller's fluid and subsequently in strong alcohol, or when they have been placed in ether and then hardened in alcohol, or when they have been entirely hardened in alcohol, the myelin is coagulated in the form of a network—the neuro-keratin network. This method also stains the neuro-keratin network distinctly. Fibers stained by this method may be kept for months in oil of cloves.

3. *Peroxide of Hydrogen for bleaching Peripheral Nerve Fibers stained with Osmic Acid.*—Osmic acid preserves nerve fibers more perfectly than any other reagent, but very often the medullary sheath is stained so dark that the other details are obscured. Peroxide of hydrogen removes the black color from the myelin and makes the fibers transparent, so that they may be examined with high powers. The stained fibers are teased in water, and a few drops of hy-

drogen peroxide are flowed under the cover glass, while the specimen is being examined with high-power lenses. Sections of osmic-acid nerves may be bleached in the same way, but it is difficult to stain them afterward with the ordinary dyes. If the fibers are treated with strong alcohol after they have been bleached in the peroxide solution, the myelin coagulates in the form of a network. This method is good for studying the incisures of Schmidt and the structure and size of the axis-cylinder, which does not shrink in careful osmic-acid hardening.

ACID FUCHSIN AND PICRIC-ACID MIXTURE FOR STAINING SECTIONS OF THE PERIPHERAL NERVES AND CENTRAL NERVOUS SYSTEM.

Sections which have been properly hardened in Müller's fluid and then in alcohol are stained rather deeply with hæmatoxylin—preferably Delafield's solution—to color the nuclei. They are then washed in water, and left for three to five minutes in acid fuchsin and picric-acid mixture prepared as follows: A few drops of a saturated aqueous solution of Grübler's acid fuchsin is added to one hundred c. c. of a saturated aqueous solution of picric acid, until the mixture has a dark-garnet color. The sections are then rapidly washed in water and in two volumes of alcohol, cleared in oil of origanum, and mounted in balsam. This stain selects the ganglion cells, neuroglia, blood-vessels, and sclerotic areas, distinctly giving them a garnet color. The axis-cylinders are stained red and the myelin is stained yellow. This stain is used considerably in the laboratory in place of carmine.

REASONS WHY

Physicians should Subscribe

— FOR —

The New York Medical Journal,

EDITED BY FRANK P. FOSTER, M. D.,

Published by D. APPLETON & CO., 1, 3, & 5 Bond St.

---

1. **BECAUSE:** It is the *LEADING JOURNAL* of America, and contains more reading-matter than any other journal of its class.
2. **BECAUSE:** It is the exponent of the most advanced scientific medical thought.
3. **BECAUSE:** Its contributors are among the most learned medical men of this country.
4. **BECAUSE:** Its "Original Articles" are the results of scientific observation and research, and are of infinite practical value to the general practitioner.
5. **BECAUSE:** The "Reports on the Progress of Medicine," which are published from time to time, contain the most recent discoveries in the various departments of medicine, and are written by practitioners especially qualified for the purpose.
6. **BECAUSE:** The column devoted in each number to "Therapeutical Notes" contains a *résumé* of the practical application of the most recent therapeutic novelties.
7. **BECAUSE:** The Society Proceedings, of which each number contains one or more, are reports of the practical experience of prominent physicians who thus give to the profession the results of certain modes of treatment in given cases.
8. **BECAUSE:** The Editorial Columns are controlled only by the desire to promote the welfare, honor, and advancement of the science of medicine, as viewed from a standpoint looking to the best interests of the profession.
9. **BECAUSE:** Nothing is admitted to its columns that has not some bearing on medicine, or is not possessed of some practical value.
10. **BECAUSE:** It is published solely in the interests of medicine, and for the upholding of the elevated position occupied by the profession of America.

Subscription Price, $5.00 per Annum. Volumes begin in January and July.